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Research Article

Bloom occurrence and phylogeny of *Gonyaulax polygramma* (Dinophyceae) isolated from south east coast of Iran (Oman Sea)

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Abstract

Harmful blooms of dinoflagellates in southeastern Iran have been increased in recent years. A dense bloom of dinoflagellate species occurred in the Ramin coast after South West Monsoon (SWM) in southeastern Iran. In order to molecular and morphological species identification of the bloom, sampling was done in October 2017, in the phycolab; the dominant bloom-forming species was isolated into a unialgal culture and kept under 12L: 12D and 25°C. The result showed the dominant bloom-forming species belonged to the genus *Gonyaulax* that co-occurred with the *Levanderina fissa*, *Scrippsiella trochoidea*, and *Prorocentrum micans* species with the lower density. Phytoplankton density was 15×10^6 cells l^{-1}. Morphological features and nucleotide sequences of the species were similar to *Gonyaulax polygramma* with 97% bootstrap support. This is the first record of a red tide incident triggered by *G. polygramma* in the northern Oman Sea, which was associated with changed water color and foam formation. This species is cosmopolitan in coastal waters, which can form harmful algal blooms (HABs).

Keywords: Dinoflagellate, *Gonyaulax polygramma*, Harmful Algae Bloom (HAB), LSU rRNA, Phylogeny

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Introduction

Algal blooms can alter the balance of food webs and cause widespread mortality of fish and shellfish. They often cause damage to the aquaculture and tourism industries of many countries. Dinoflagellates are one of the most important groups of phytoplankton, many of which have the potential to form blooms and can cause harmful algal blooms (Hayes *et al*., 2008). The rapid growth of certain dinoflagellate cells (more than a million cells per liter) and water discoloration called an algal bloom, if this bloom damages organism or ecosystem commonly referred to as Harmful Algae Bloom (HAB) (Hallegraeff, 2004). There are many reasons for the worldwide spread of harmful phytoplankton species. That is human activities such as discharge of industrial effluents into the water, nutrients from pools, rainfall, and wind stresses, which facilitate bloom.

Oman Sea has a sub-tropical climate and is exposed to annual monsoon winds. In the Oman Sea and the Persian Gulf, phytoplankton blooms are reported every year. A record of over 40 microalgae blooms events have been observed in the Persian Gulf and the Oman Sea from 1991 to 2012, most of which were caused by species such as *Trichodesmium* sp., *Noctiluca scintillans* (Macartney) Kofoid and Swezy, *Nitzschia* sp., *Gonyaulax* sp., *Karenia selliformis* Haywood, Steidinger et MacKenzie, Gymnodinium, *Prorocentrum micans* Ehrenberg, (Rohani-Ghadikolaei *et al*., 2012; Thangaraja *et al*., 2007). Additionally, harmful booms caused by *Cochlodinium polykrikoides* Margalef were also reported during 2008-2009 in the Persian Gulf and the Oman Sea that lasted more than eight months and 34 tons of marine organism's mortality occurred, causing damage to tourism and fisheries (Richlen *et al*., 2010; Al-Azri *et al*., 2014). After the *Cochlodinium polykrikoides* bloom, precise identification of bloom-forming species became a priority in all the coastal countries such as Iran in the Oman Sea and the Persian Gulf regions. Since 2009, most blooms that occurred in the region have been related to the genera *Cochlodinium*, *Gonyaulax*, *Karenia*. It is worth noting that the bloom *of C. polykrikoides* was repeated in the Persian Gulf in 2010, but without any fish mortality (Zarshenas *et al*., 2014). 22 phytoplankton species have the potentials to form harmful blooms in the north part of the Oman Sea (Attaran-Fariman and Sharifian, 2014; Attaran-Fariman 2016). *Gonyaulax* currently consisted of 128 species with seven infra-specific names in AlgaeBase; however, only 76 species names are accepted taxonomically (Guiry and Guiry, 2020). The genus is widespread and distributed in the freshwater, brackish, lake, and marine waters in temperate and tropical areas. There are some records of red tide for *Gonyaulax polygramma* Stein from different geographical areas around the globe. Although the species is not toxic, it has the potential to cause harmful algal blooms. A bloom of *G. polygramma* in

[DOR: 20.1001.1.15622916.2021.20.6.16.5]

1994 in Uwajima Bay caused fish and shellfish mortality, as well as economic losses of 800 million Yen in south Japan (Koizumi *et al*., 1996). Subsurface blooms of this species were also reported from coastal waters of the eastern Arabian Sea in 2008 (Al-Azri *et al*., 2012; Padmakumar *et al*., 2018). However, there is no record of bloom of this species in the northern Oman Sea, but some HAB records from the Oman Sea are attributed to *Gonyaulax* sp., with the species identification unknown. In addition, the phylogeny and molecular identification of this HAB species from the Oman Sea have not been evaluated. Many dinoflagellate species formed blooms, produce resting cyst during their life cycle, settle on the sediment and act as a seed bank to initiate the bloom (Attaran-Fariman, 2007; Attaran-Fariman et al., 2012). There are not any researches on the cyst formation of *Gonyaulax polygramma* in the literatures.

Species identification solely on morphological features is often challenging and frequently depends on cultures (Dodge, 1989). With the careful identification of causative bloom-forming species and a better understanding of the biogeography of the species, we have established a unialgal culture from the isolated single cell of the bloom area waters for molecular analysis and assess the formation of a cyst. This study aims to investigate the occurrence, morphology, and phylogeny inferred based on LSU-rDNA of species that caused red tide in 2017 in the Southeast coast of Iran.

Material and methods

Sampling and culture

Water sampling was performed during the bloom of dinoflagellate species on 20, 23 October 2017 in the southeastern of the Oman Sea (Fig. 1). One liter of brown-colored surface water was collected (25° 15.21′ N, 60º 44.40′ E).

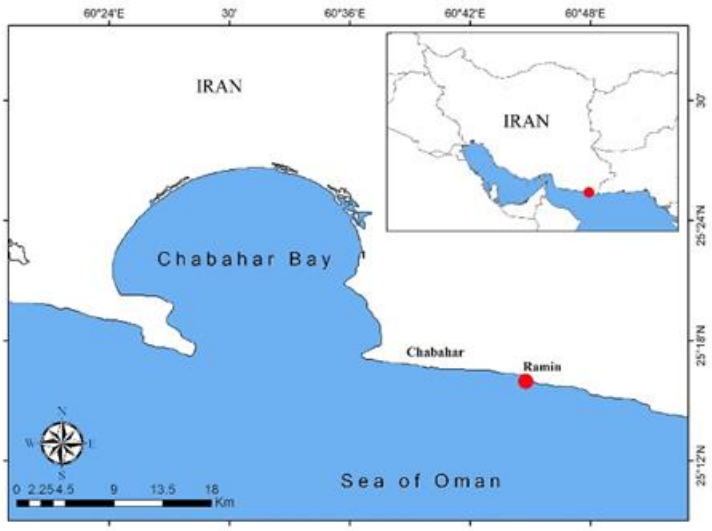

Figure 1: Map of the bloom area (Ramin coast) in the southeast coast of Iran.

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Water temperature and salinity were determined simultaneously, at the sampling site. The water sample was immediately transferred to the laboratory, to establish a clonal culture of the causative red tide species. Single cells were isolated (with more than 10 replications) into plate containing F/2 culture medium by micropipette under Nikon-TF100 inverted microscope (Nikon, Tokyo, Japan) and incubated at 12:12 (L:D), at 25°C±1 and irradiance of 60 µmol photons m-2 sec-1provided by white fluorescent light. One milliliter of clonal culture was placed in a Petri dish containing 10 ml of F/2 medium without nitrate and phosphate to check cyst formation of the bloom formed species. Morphological features were assessed based on literature (Guillard and Ryther, 1962; Kim *et al*., 2005) with an inverted microscope equipped with a digital camera (D5600 Nikon, Japan). The established cultures (strain; CHPGp1) were deposited in the culture collection of Chabahar Maritime University in Iran.

DNA Extraction and phylogenetic analyses

DNA of the unialgal *G. polygramma* strain (CHPGp1) was extracted by the CTAB method (Hansen *et al*., 2003; Simonelli *et al*., 2009; Wylezich *et al*., 2010) and suspended in 100 ml Tris-EDTA buffer. Evaluation of DNA quality was observed on 1% agarose gel staining with ethidium bromide using electrophoresis (PNP-1000d, Iran) and Gel Doc (E-BOX-VX2/20M, Germany) imaging system, and quantity was verified using a spectrophotometer (Model, RS232C, China). DNA is used as a template in the PCR reaction to amplify partial large subunit LSU rRNA gen. PCR primers D1R (5´-ACCGCTGAATTTAAGCATA-3´) (Scholin *et al*., 1994) and 1483R (5´-CTACTACCACCAAGATCTGC-3´) (Daugbjerg *et al*., 2000) were used to amplify partial 28S rRNA genes. PCR reaction was done in 50 µL volume of 5 ng DNA, 3.5 µmol Mgcl2, 2U Tag, 200 µmol dNTP, and 10 picomols of each primer. The PCR program used included an initial separation at 94°C for 2 min, 35 cycles, 94°C for 45 sec, 56°C for 50 sec, 72°C for 1 min and the final expansion temperature was 72°C for 4 mi. The PCR product was quantified using electrophoresis with 1% agarose gel and observed on Gel Doc (E-BOX-VX2/20M, Germany). The PCR product was sequenced. The gene sequence of *G. polygramma* strain CHPGp1 obtained in the present study was deposited to the GenBank database under ascension number MK321262.

Sequences of partial LSU rRNA obtained from the present study with the sequence of 29 species belonging to Dinophyceae available in GenBank were aligned using ClustalW (Thompson *et al*. 2003) and improved manually in BioEdit (Hall, 1999). Neighbor-joining (NJ) and maximum parsimony (MP) analyses were used to evaluate the phylogeny of the species using Mega 7 (Tamura *et al*., 2011).

[DOR: 20.1001.1.15622916.2021.20.6.16.5]

Results

Examination of the bloom

A dense algal bloom occurred in south-eastern Oman Sea and spread over an area 30 × 4 km in coastal waters from 20 to 23 October 2017, lasting four days and the watercolor had been turned to brown (Fig. 2) with the formation of foam.

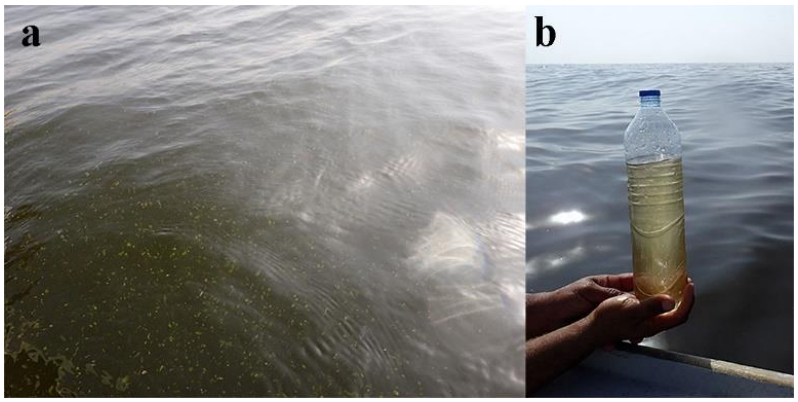

Figure 2: Brown watercolor caused by *Gonyaulax polygramma* bloom in the Oman Sea 2017.

The water sample showed a high density of phytoplankton with 15×10^6 cells l^{-1}in the first day of bloom and on the fourth day the frequency of species decreased significantly (1.1×10^6 cells L^{-1}). The predominant phytoplankton species was *G. polygramma* (Fig. 3a-l); however, several dinoflagellates including *Levanderina fissa* (Levander) Moestrup, Hakanen, Gert Hansen, Daugbjerg and M.Ellegaard, *P. micans*, *Prorocentrum dentatum* Stein, and *Scrippsiella trochoidea* (Stein) Loeblich III increased in the incubated water sample in the lab simultaneously after few days. This bloom created foam in the area with no apparent marine organism mortality. Sea surface temperature in the bloom area was 29°C±1 and salinity 36 PSU.

Species identification

Motile and empty cells of *G. polygramma* were present in the culture medium (Fig. 3a-b). The light brown *G. polygramma* cells were 37–45 µm long and 28–38 µm wide with a triangular epitheca and a round hypotheca (Fig. 3d-e). The nucleus was in the hypotheca (Fig. 3d). In the motile cells, pyrenoids and round chloroplasts were at the surface focus (Fig. 3e). The antapex was associated with two, small, spine-like appendages (Fig. 3f-h). The thecal plates were relatively thick with longitudinal stria. The cingulum displacement was 2 times of cingulum width. The species was identified as *G. polygramma* based on morphology (Fig. 3f). Temporary cysts of *G. polygramma* were produced in the F/2 culture medium without nitrate and phosphate and also in the old culture medium (Fig. 4a-b).

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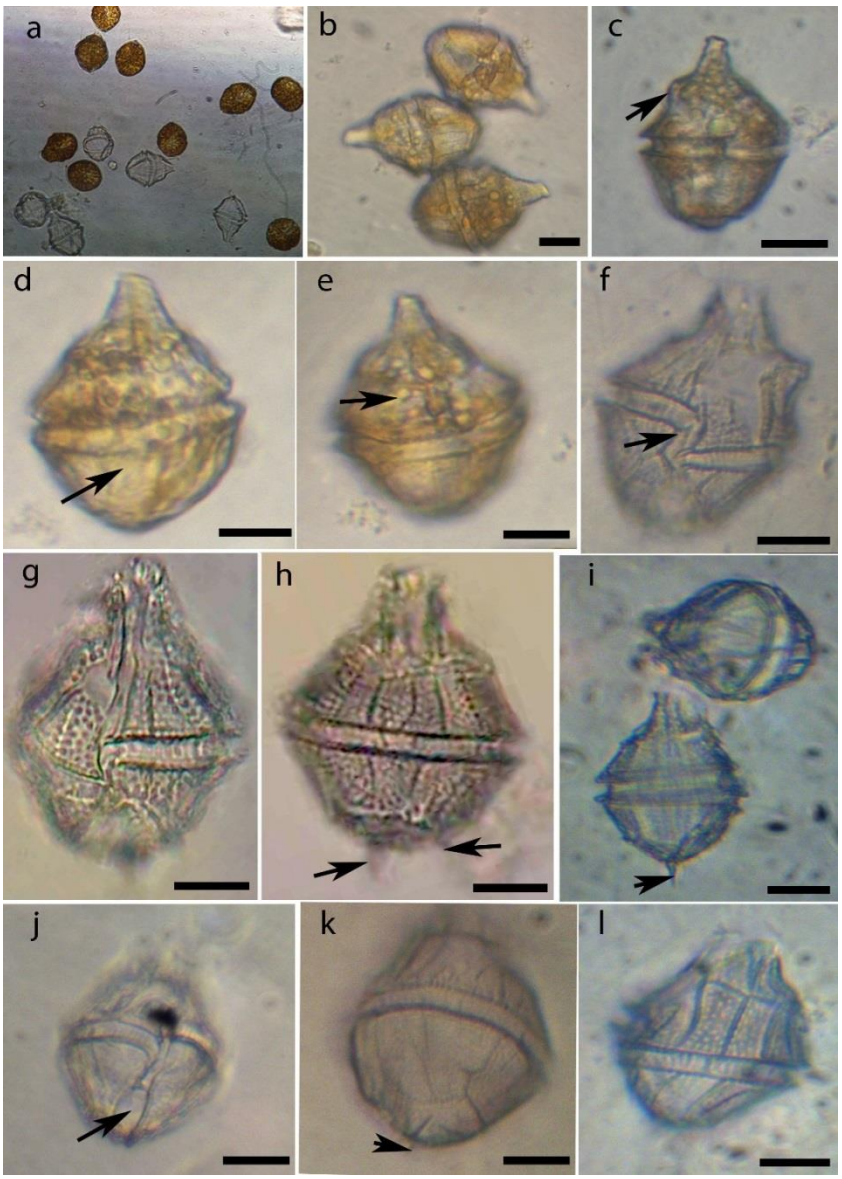

Figure 3: *Gonyaulax polygramma* (strain CHPGp1), isolated in October 2017 from the southeastern coast of Iran. Scale bars=10 µm (a. Vegetative and empty cells in culture, b-d. Vegetative cells; dorsal of cell showing shoulder of epitheca and nucleus (arrow) in hypotheca, e. Vegetative cells with a pyrenoid (arrow) in surface focus, f-h. Ventral of the empty cell shows cingulum displacement and reticulate cell surface, h-i. Lateral and dorsal of empty cells with antapical spins (arrows), j. Empty cell showing sulcus (arrow), k. Cells in antapex showing spine and antapical plate pattern, l. Dorsal of the cell showing plate pattern).

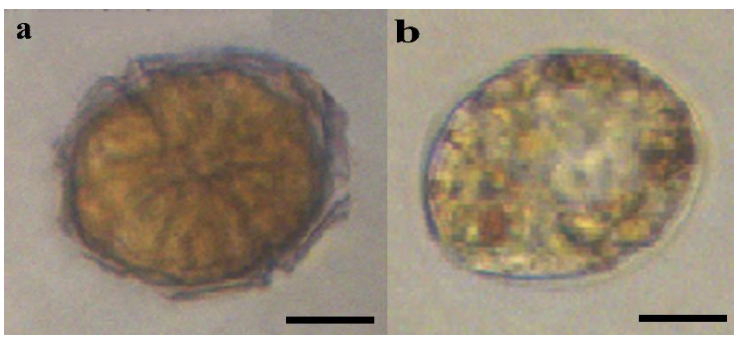

Figure 4: Temporary cyst of *G. polygramma* (strain GHPGp1).

[DOR: 20.1001.1.15622916.2021.20.6.16.5]

A red accumulation body occurred in the cyst.

Molecular analysis was performed using 29 species of Dinophyceae including representatives of orders Gymnodiniales, Gonyaulacales, Prorocentrales, Thoracosphaerales based on partial sequences of the D1-D6 regions of the LSU-rDNA. Partial sequences of the LSU-rDNA gene and maximum parsimony analysis generated a tree shown in Fig. 5.

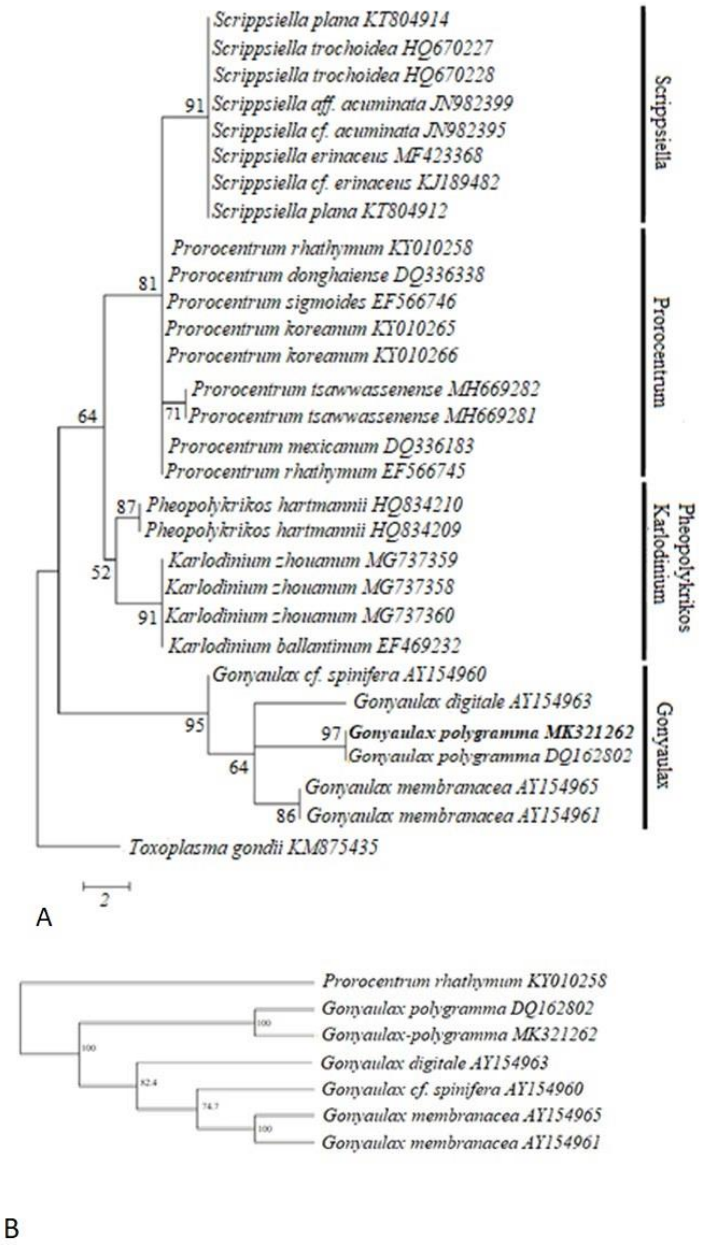

Figure 5: Phylogenetic tree of *Gonyaulax polygramma* CHPGp1 strain isolated from Oman Sea based on partial LSU rRNA using: (top tree) Maximum Parsimony and Replication 1000 of the full heuristic search algorithm. *Toxoplasma gondii* Nicolle and Manceaux (KM875435) was used as the outgroup, Maximum Composite Likelihood test (bottom tree) *Gonyaulax spp* NJ tree.

[DOR: 20.1001.1.15622916.2021.20.6.16.5]

The present phylogenetic tree has 4 clades (Fig. 5); Clade A contains *Scrippsiella plana* Luo, Mertens, Bagheri et Gu, *Scrippsiella cf. acuminate* (Ehrenberg) Kretschmann, Elbrächter, Zinssmeister, Soehner, Kirsch, Kusber et Gottschling, *Scrippsiella erinaceus* (Kamptner) Kretschmann, Zinssmeister et Gottschling and *S. trochoidea* has 91% Bootstrap support. This clade has a polyphyletic relationship with clade B and a monophyletic relationship with clade D. Clade B contains different strains of Prorocentrum: *Prorocentrum tsawwassenense* Hoppenrath & Leander, *Prorocentrum sigmoides* Böhm, *Prorocentrum koreanum* Han, Cho et Wang, *Prorocentrum mexicanum* Osorio-Tafall, *Prorocentrum rhathymum* Loeblich III, Sherley et Schmidt, *Prorocentrum donghaiense* Lu, supported by 81% Bootstrap. This Clade has a monophyletic relationship with clades C and D. Clade C includes *Pheopolykrikos hartmannii* (Zimmermann) Matsuoka et Fukuyo, *Karlodinium zhouanum* Luo & Gu, and *K. ballantinum* de Salas. Molecular and phylogenetic analyses revealed that the Iranian strain is in clade D of MP tree in Gonyaulacales, and this clade includes *G. polygramma*, *G. digitale* (Pouchet) Kofoid, *G. membranacea* (Rossignol) Ellegaard, Daugbjerg, Rochon, Lewis et Harding, and *G. cf. spinifera* (Claparède & Lachmann) Diesing supported with 95% Bootstrap. The species responsible for the bloom in Iran's south coast was allied with *G. polygramma* (97% supports) from Korea (Fig. 5). This clade has a monophyletic relationship with clade D. *Toxoplasma gondii* is considered as an outgroup. *G. polygramma* from the Oman Sea is allied with Korean strain with 100% support only in the Gonyaulacales tree (Fig. 5).

Discussion

According to our results, *G. polygramma* caused bloom in October 2017 after the South West Monsoon (SWM) in the northern Oman Sea. The Oman Sea is a tropical region located in the northern Indian Ocean, which lies between the Arabian Sea and the Persian Gulf. During SWM (June-September), strong winds blow from the Indian Ocean to the Oman Sea causing monsoon currents. Upwelling transfer nutrient-rich water from depth to the surface during the monsoon season in the Oman Sea. The change in phytoplankton species composition and density, in all tropical regions after the SWM, is a common phenomenon (Kimor, 1992; Esparza-Alvarez *et al*., 2007; Baliarsingh *et al*., 2018). Most plankton blooms occur in different parts of the Indian Ocean and the Oman Sea after SWM. Monsoon winds and turbulent mixing upwelling enhance nutrient recharge in the surface water (Pautova *et al*., 2017), and *G. polygramma* can migrate to this nutrient-enriched water layer. *G. polygramma* is a cosmopolitan species and distributed from neritic to oceanic, and temperate to tropical areas (Cho, 2011). Temperature cannot limit this alga because it has a wide temperature tolerance (Hasle *et al*., 1996). In addition, low rainfall and low

[DOR: 20.1001.1.15622916.2021.20.6.16.5]

water exchange rates can also cause blooms of this species. In the present study, the water temperature at the time of bloom was 29ºC. The surface water temperature was 29.5ºC in the bloom of *G. polygramma* in the southeast Arabian Sea (Padmakumar *et al*., 2018) and 25ºC in the South Sea in Korean waters (Cho, 2005). It seems that the water temperature above 25ºC is more suitable for the bloom of this species. The species is normally considered a warm water species and is widely distributed in the Indian Ocean. The first bloom of this species was reported from Japanese waters and resulted in extensive oyster mortality in 1900 (Nishikawa, 1901).

The species is recorded in the Oman Sea and the Arabian Sea. Blooms of this species have been occurred several times in different years in the Arabian Sea and southern Oman Sea (Fig. 6), but it was first spread in the northern Oman Sea in October 2017. *G. polygramma* is potentially a harmful bloom-forming species and has frequently caused red tides leading to marine organism mortality in many areas (Table. 1). Blooms of *G. polygramma* were reported previously from Omani waters (Al-Ghelani *et al*., 2011), with four bloom incidents caused by *G. polygramma* occurring in the southern Oman Sea. These blooms were associated with fish mortality, but the authors did not provide the blooming year. According to the documents *G. polygramma* is nontoxic, it can cause aquatic organism mortality due to low dissolved oxygen (Cho, 2005; Kumar *et al*., 2020), however, toxicity test has not yet been done on the Iranian strain. The blooming of *G. polygramma* in Uwajima Bay in Japan caused mass deaths of fish and shellfish, and the water was brown and stinky due to lack of oxygen. Degradation of *G. polygramma* caused an increase in sodium and ammonium in the water (Koizumi *et al*., 1996). *G. polygramma* migrates daily into the superficial layer in the day and lower layers at night (Koizumi *et al*., 1996). The species caused a red tide without fish mortality in Korea in 2009, the one month bloom duration of this species was significant and contrasts with the Iranian bloom which lasted only four days. The bloom of this species is not usually monospecific and is associated with other dinoflagellates and diatoms. Other dinoflagellate species in the bloom sample included *G. instriatum* (8×104 cells l-1), *S. trochoidea* (2.5×105 cells l-1), and *P. micans* (2.2×105 cells l-1) were noted. Diatoms included *Odontella aurita* (Lyngbye) Agardh (1.1×104 cells l-1), *Hanitzschia sigma* (Kützing) W.Smith (1×104 cells l-1), *Tabellaria sp*. (8×103 cells l-1), and *Leptocylindrus danicus* Cleve (3.3×104 cells l-1). Among the dinoflagellates observed in the bloom region, it was noteworthy that most species had the potential to form blooms, but only *G. polygramma* became dominant. In the Gulf of South Africa, Grindley *et al*. (1964) noted that a bloom of *G. polygramma* was associated with other dinoflagellates in low density, with *P. micans* being the most common.

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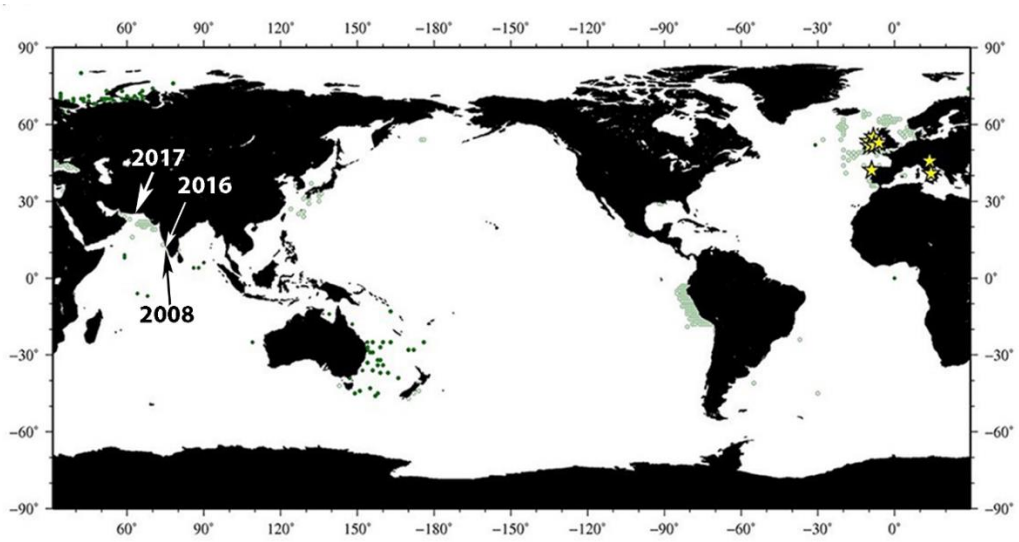

Figure 6: Global distribution of *G. polygramma* (in green) with years of red tide events in the northern Indian Ocean (adapted from https://www.st.nfms.noaa.gov/copepod,2019).

Table 1: Historical records on the occurrence of HABs of *Gonyaulax Polygramma.*

Area	Period	Number of cells (cells l^{-1})	Mortality	Source
False bay (South Africa)	Mar., Apr. 1962	-	Fish, invertebrates	Grindley *et al.*, 1964
Oman (south part of Oman sea)	1976	-	Fish	Barwani , 1976
Uwajima bay (Japan)	Aug., Nov. 1994	6.8×10^7	Fish, shellfish	Koizumi *et a.*, 1996
Douglas Cay (Central America)	May 1995, 1996	3.5×10^6(1995), 1.8×10^6(1996)	-	Morton & Villareal , 1998
Korea, Tongyeong	2004	-	-	Keun-Yong Kim *et al.*, 2006
Korea, Yeosu (South Sea)	Aug. 2004	2×10^6 to 4×10^6	-	Cho , 2005
Mangalore (Arabian Sea)	Oct. 2008	5×10^8	Zooplankton	Padmakumar *et al.*, 2018
Korea southern coast	Summer, 2009	6.5×10^6 to 12.5×10^6	-	Cho , 2011
Caspian Sea	Summer 2010, 2013	-	-	Pautova *et al.*, 2017
Southern Benguela (African)	2011	-	Fish	Van der Lingen *et al.*, 2016
Gulf of California	Sep. 2012	-	-	Gárate-Lizárraga *et al.*, 2014
Puri, Odisha (east coast of India)	May 2016	$1.62 \pm 0.8 \times 10^4$	-	Baliarsingh *et al.*, 2018
Kochi (Arabian Sea)	Nov. 2014, 2015, 2016	4.9×10^6	-	Kumar *et al.*, 2020
South of the Oman	-	-	Fish	Al Gheilani *et al*., 2011
Northern part of Oman Sea	Oct. 2017	15×10^6	-	current study

In the southeast Arabian Sea, in the *G. polygramma* bloom in 2016, other dinoflagellates including *Protopredinium spp*, *Noctiluca scintillans*, and *Ceratium spp*, and the diatoms *Rhizosolenia spp*., *Asterionella*

[DOR: 20.1001.1.15622916.2021.20.6.16.5]

sp., and *Chaetoceros spp*, occurred in low densities (Padmakumar *et al*., 2018). Van der Lengen (2016) documented a bloom in 2011 from South Africa in which *G. polygramma* was the dominant species and was associated with other dinoflagellate and diatoms species. The first bloom of this species was in the north of the Oman Sea and the first molecular identification of this species was done in the Persian Gulf and the Sea of Oman. The bloom of this species with 15×10^6 cells l^{-1} changed the color of the water, created a bad smell, and foam in the area.

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